

## Use of Adjuvants for Enhancement of Rectal Absorption of Cefoxitin in Humans

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**The biological availability of cefoxitin administered rectally in the form of suppositories was examined in six human subjects by a cross-over design. Four different suppository systems containing adjuvants expected to enhance the absorption of the drug were studied. The presence of sodium salicylate and a nonionic surface-active agent, Brij 35, gave increased bioavailability as high as 20% compared with 3% for a system without adjuvants. The quantity of sodium salicylate was found to have an influence on the quantity of cefoxitin absorbed, and the salicylate was absorbed over an extended period of time from the rectum. The suppositories were well tolerated, and there were no adverse effects on bowel flora.**

The rectal route of drug administration has certain advantages over the more popular oral route and can be especially useful in pediatric patients and in those with certain disease conditions, for example in patients with epilepsy and arthritis. The rectal mucosa may offer a more reliable and well-defined region for drug absorption than does the small intestine; in addition, the rectal administration of drugs may lead to a reduction in the initial metabolic destruction by the liver after absorption from the intestine (first-pass effect).

Results of studies in animals (rats and more recently dogs) have shown that certain innocuous pharmaceutical adjuvants (namely, salicylates, benzoates, and related compounds) can lead to enhanced absorption of drugs, particularly those that are soluble in water, such as theophylline, lidocaine, levodopa, cephamycin antibiotics, gentamicin and penicillin G (3-14; J. Fix, L. J. Caldwell, J. Haslam, P. A. Porter, K. Engle, P. S. Leppert, R. Schaffer, L. Frost, A. Mlodozieniec, and T. Higuchi, manuscript in preparation).

The adjuvant and drug need to be administered concurrently, and histological examinations have failed to reveal any adjuvant-induced changes in the mucosa (8). The mechanism of the adjuvant effect has not yet been fully elucidated but could involve the passage of drug molecules through the tight junctions of cells as well as possible involvement of the lymphatic system (8). The roles of calcium and magnesium, as well as ionic strength, have been discussed (3, 14).

The enhanced absorption for the cephamycin antibiotics cefmazozone and cefoxitin (13, 14; Fix et al. in preparation) is particularly interesting because at present both agents are given by intravenous (i.v.) or intramuscular (i.m.) injection. A well-absorbed rectal formulation would be an advantage, particularly as a means of reducing muscle damage caused by repeated injections (14). This paper describes the evaluation of cefoxitin suppositories containing absorption-enhancing adjuvants in humans. The effects of different quantities of drug and adjuvant were studied as were any changes to the bowel flora. Sodium salicylate and the nonionic surfactant Brij 35 [polyoxy-ethylene(23)lauryl ether] were selected as the combined adjuvant because of the results of studies of these adjuvants in rats and dogs (4; Fix et al., in preparation) with microenemas and lipophilic suppository bases. Sodium salicylate has been administered

rectally to humans by microenema and suppositories at concentrations up to 20% or as 0.5-g doses in suppositories (5, 15, 16). Brij 35 surfactants previously have been administered rectally to humans at low concentrations without adverse reactions (1). Higher concentrations of nonionic surfactants are known to cause histological changes that can be associated with an increase in permeability in the rectal tissue of rats (6).

### MATERIALS AND METHODS

**Suppository preparation.** Sodium salicylate (quality as established in the British Pharmacopeia; Thornton Ross) was micronized and then passed through a 90- $\mu$ m mesh sieve. Sodium cefoxitin (Merck Sharp & Dohme) was passed through the same sieve. Brij 35 (Atlas) was a gift from Honeywell-Stein, London. The suppository base, Witepsol H-15 (Dynamit-Nobel), was used as received.

The Witepsol H-15 base was melted over a water bath, the components were stirred into the molten mass, and the system was filled into plastic molds to give a total weight of 3.3 g for each suppository (Table 1). The suppositories were stored at 4°C before use.

**Analytical procedure.** A high-pressure liquid chromatographic method was used to measure the levels of cefoxitin in serum and urine (2, 14, 17; Fix et al., in preparation). A C-18 Partisil column containing a 10- $\mu$ m particle-sized reversed-phase system was used. The solvent was a mixture of 25% acetonitrile-0.5% acetic acid-74.5% 0.005 M aqueous sodium dihydrogen phosphate. The presence of cefoxitin was determined at 238 nm for serum samples and at 254 nm for urine samples. To each 100- $\mu$ l serum sample was added 100  $\mu$ l of internal standard solution (aqueous cefmazozone [15  $\mu$ g/ml] prepared daily), and then 75  $\mu$ l of 10% trichloroacetic acid was added to precipitate the protein. The samples were blended with a Vortex mixer and allowed to stand, and the supernatant was injected onto the column. A standard curve for the assay series was generated by spiking blank serum with appropriate levels of cefoxitin and then processing as described above. The assay method was reproducible and specific for cefoxitin. Cefoxitin is only slightly metabolized under the dosing conditions described previously (17). The sensitivity was about 0.1  $\mu$ g/ml. Recovery of cefoxitin was about 90%, and the coefficient of variation on five replicate analyses at 1.0  $\mu$ g/ml was 1.5%. Full details have appeared

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TABLE 1. Formulations employed for studies on cefoxitin bioavailability from suppositories

Study	Sodium cefoxitin (g)	Sodium salicylate (g)	Brij 35 (g)	Comments
1	1.0			Solution in 10 ml of water for injection i.v. dose
2	1.05			
3	1.05	0.50	0.075	Two suppositories were administered
4	1.05	0.50	0.075	One suppository was administered
5	1.05	0.15	0.075	
6	0.26	0.30	0.075	

elsewhere (14; Fix et al., in preparation). The levels of sodium salicylate in blood samples were also determined by the same high-pressure liquid chromatographic procedure by first mixing 10 ml of serum with an equal volume of 5% aqueous trichloroacetic acid followed by centrifugation at 10,000 rpm for 15 min.

Levels of sodium salicylate in urine samples were determined by a method described previously (19).

**Human experiments.** Six healthy human subjects ranging in age from 23 to 29 and in body weight from 55 to 92 kg participated in the study with informed consent. The study protocol was approved by the local ethical committee. No drugs, including analgesics or laxatives, were taken 14 days before the start of the study and until the last blood sample had been taken at the end of the study. The subjects were allowed to eat and drink normally before and during the study, and they followed a similar dietary pattern for each experiment. At least 6 days were allowed to elapse between each experiment. Each subject received each of the various formulations according to a double-blind, randomized design. Injections were administered i.v. over a period of 3 to 5 min. Blood samples were taken at intervals of up to 360 min with an indwelling cannula and centrifuged immediately, and the serum samples were deep frozen before analysis. Total urine samples were collected at intervals over a 24-h period. Stool samples were collected immediately before treatment and then at 24 h and 3 to 5 days after treatment. The stool samples were refrigerated and examined the following day for cefoxitin-resistant organisms by using suitable aerobic and anaerobic culture media and by determining whether there was growth within a zone of inhibition produced by a 30- $\mu$ g cefoxitin disk. All samples were also cultured under conditions to isolate *Clostridium difficile* by using appropriate controls. Any unformed stool samples were also cultured for *Salmonella*, *Shigella*, and *Campylobacter* species.

## RESULTS

**Cefoxitin levels.** The data of levels of cefoxitin in serum obtained after i.v. administration are shown in Fig. 1 as the mean  $\pm$  standard deviation. The pharmacokinetic profile can be well described by a two-compartment open model with a terminal half-life of the order of 60 min. Results of previous studies in which single 1-g doses were administered over short time intervals, also demonstrate a terminal half-life between 40 and 60 min and similar levels of cefoxitin in serum (2, 18). The apparent volume of distribution for the central compartment in the model is about 8 liters. The same value has been reported by Schrogie et al. (17). The total area under the serum level-time curve (AUC), extrapolated

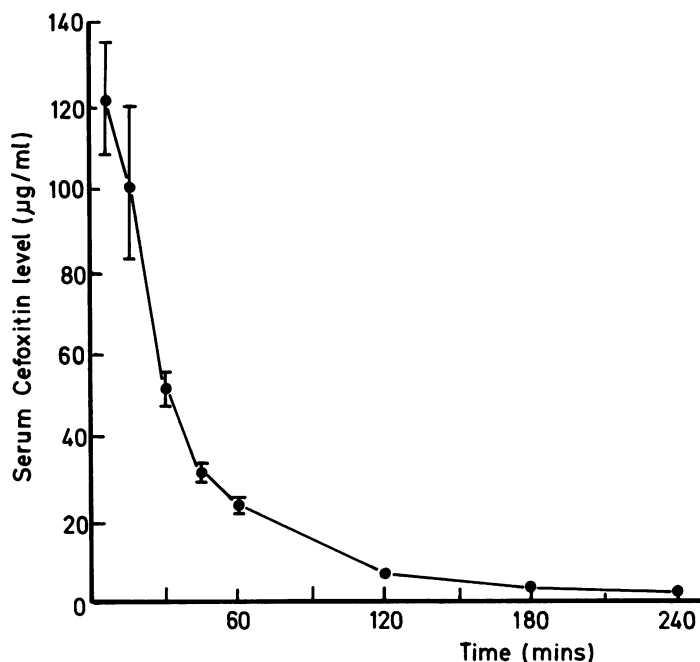


FIG. 1. Cefoxitin levels in serum after i.v. administration of a 1.0-g dose.

to infinity by fitting the data to a two-compartment model, was (mean  $\pm$  standard error of the mean)  $5,135 \pm 243$  ( $\mu$ g min  $\text{ml}^{-1}$ ).

The levels of cefoxitin in serum obtained in the different studies are shown in Fig. 2 for selected time intervals up to 360 min postadministration as the mean  $\pm$  standard deviation. It should be noted that one volunteer expelled suppository systems in studies 3 and 5 at 52 and 62 min, respectively, when the suppositories were at an advanced state of dissolution.

The data were analyzed to provide the AUC from time zero to infinity and statistical evaluation in the form of paired student *t* tests and analysis of variance. The values for the total areas are given in Table 2.

The formulation in study 2 without adjuvants had poor bioavailability. The addition of sodium salicylate and Brij 35 gave increased bioavailability for the drug. Significant differences between the treatments in terms of the levels in serum

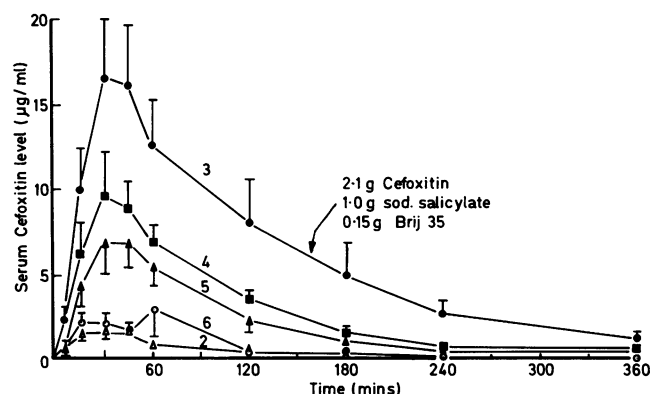


FIG. 2. Cefoxitin levels in serum after administration of suppository formulations. Symbols:  $\Delta$ , study 2;  $\bullet$ , study 3;  $\blacksquare$ , study 4;  $\blacktriangle$ , study 5;  $\circ$ , study 6.

TABLE 2. Pharmacokinetic data for the rectal administration of cefotitin in the presence of adjuvants<sup>a</sup>

Study	Cefotitin dose (g)	Peak level in serum ( $\mu\text{g/ml}$ )	AUC	Relative bioavailability (%) in <sup>b</sup> :		Sodium salicylate dose (g)	Sodium salicylate recovered in urine at 0 to 24 h (mg)
				Serum	Urine		
1 (i.v.)	1.0	122.5 $\pm$ 13.6	5,135 $\pm$ 243	100	100		
2	1.05	1.8 $\pm$ 0.40	174 $\pm$ 33	3.2 $\pm$ 0.6	4.4	0	0
3	2.10	16.7 $\pm$ 3.3	2,330 $\pm$ 582	21.6 $\pm$ 5.4	37.5	1.0	590 $\pm$ 311
4	1.05	9.8 $\pm$ 2.3	1,162 $\pm$ 193	21.5 $\pm$ 3.6	21	0.5	225 $\pm$ 69
5	1.05	6.8 $\pm$ 1.9	666 $\pm$ 128	12.3 $\pm$ 2.4	15	0.15	61 $\pm$ 20
6	0.26	1.2 $\pm$ 0.36	183 $\pm$ 42	12.3 $\pm$ 3.0	19	0.30	87 $\pm$ 28

<sup>a</sup> Data are expressed as mean  $\pm$  standard error of the mean.

<sup>b</sup> Relative bioavailability for serum data was obtained by the following equation: percent bioavailability =  $(AUC)_{\text{rectal}} \times (\text{dose})_{\text{i.v.}} \times 100 / (\text{dose})_{\text{rectal}} \times (AUC)_{\text{i.v.}}$ .

at 30, 45, and 60 min and peak levels and AUC were obtained, such that study 2 was significantly different than studies 3, 4, and 5. Study 3 was significantly different than studies 2, 4, 5, and 6. Study 4 was different than studies 2, 3, and 6, and study 5 was significantly different than studies 2, 3, and 6.

Two suppositories (study 3) produced approximately twice the effect of one suppository (study 4) of the same composition. Peak levels of cefotitin were obtained after 30 min for all studies except study 6. Marked intersubject variation was observed, but the individual subjects all provided data that ranked the studies in the order  $3 > 4 > 5 > 6 = 2$ . In addition, an individual who demonstrated good absorption for one formulation of the series, as compared with other members of the same study group, consistently absorbed all other formulations well. The relative bioavailabilities expressed in terms of the AUC extrapolated to infinity as compared with the data obtained for i.v. administration normalized for the dose of cefotitin are given in Table 2. The dramatic effect of the adjuvants is well demonstrated. The quantity of salicylate in the study is also a factor in determining the bioavailability of cefotitin.

A comparison of the cefotitin concentration in serum-time profiles after separate administration of sodium cefotitin to human subjects by i.m. injection (data on file, Merck Sharp & Dohme Laboratories) and by suppositories in studies 3 and 4 of the present work) is shown in Fig. 3. It is clear that

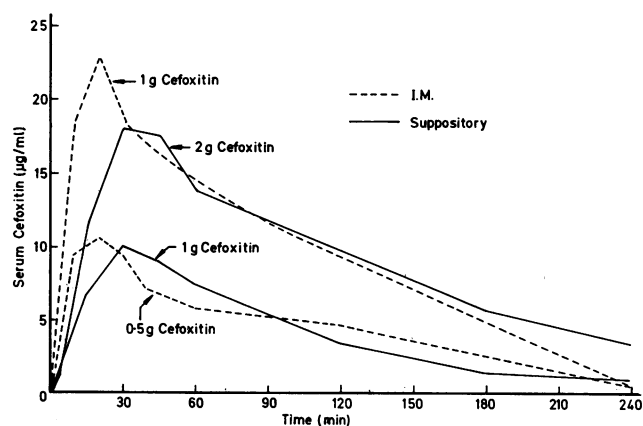


FIG. 3. Cefotitin levels in serum after administration by i.m. injection (data from reference 15 and Merck Sharp & Dohme Research Laboratories) and suppository formulations (studies 3 and 4; suppository data for five subjects; data for the individual who expelled the suppository in study 3 during the study period are omitted).

by increasing the dose by a factor of two in the rectal dosage form it is possible to almost duplicate the profiles obtained by i.m. administration, although the rate of absorption for the suppository system is somewhat slower than for the i.m. system.

Complete urine level data were available from 24-h collections for all volunteers for at least five or all six studies. The comparative bioavailabilities based on total recovery of cefotitin are given in Table 2. The agreement between data on serum and urine levels is generally good, although study 3, comprising two suppositories, would indicate an improved bioavailability as compared with study 4 for the same composition, but only one suppository was administered. This difference is associated with the problems inherent in total collections of urine from all subjects over a 24-h period for all studies.

**Salicylate levels.** The salicylate determinations in serum and urine showed that the adjuvant was absorbed from the rectum along with the drug. The salicylate levels in serum were in accord with the doses of salicylate administered in the various formulations. However, for analytical reasons complete data for salicylate were not available for all individuals and all studies. Figure 4 shows salicylate levels for studies 3 and 4 obtained from data on 5 and 4 subjects, respectively. It should be noted that the absorption phase for sodium salicylate is much longer than that for cefotitin, and for study 3 the peak level occurs between 2 and 3 h after administration. In contrast, the cefotitin peaks occurred at 30 min (except for study 6). The peak levels of salicylate in Fig. 3 are approximately in the ratio of the doses of salicylate administered.

The salicylate data also showed much greater variation than the corresponding values for cefotitin. Similar plasma level-time profiles have been presented by Schoonen and co-workers (5, 16), and their pooled data ( $n = 4$ ) for Witepsol H-15 suppositories (no size given) containing 11.6 and 23.2% sodium salicylate are plotted in Fig. 3. A similar long absorption phase and variability is demonstrated in the present work. Schoonen and co-workers have discussed the importance of viscous drainage and particle size effects in determining the release of water-soluble drugs from fatty suppository bases (5, 16).

The recovery data of urine also demonstrated variability, but the mean values ( $n = 6$ ) were in proportion to the doses of salicylate given (Table 2). (The expulsion of the suppository part way through the study by one individual was a contributory factor to the variability in some of the results.)

**Clinical observations.** The suppository formulations were well tolerated by the volunteers, and there were no reports of irritation. All subjects reported flatus. Four subjects reported tenesmus, usually within 30 min. Three subjects

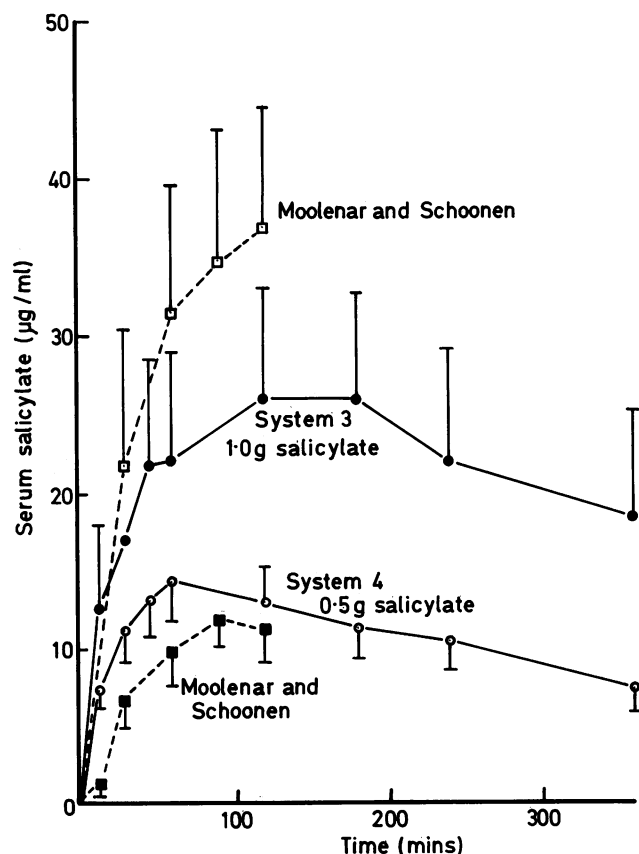


FIG. 4. Salicylate levels in serum after administration of suppository formulations. Symbols: ●, study 3; ○, study 4. Data obtained from reference 12. Suppositories contained 11.6% (■) and 23.2% (□) sodium salicylate.

reported abdominal discomfort and nausea. In one case this was associated with a recent gastrointestinal disturbance. Two of these individuals were tested further with a range of suppositories of different formulations to provide a range of adjuvant combinations. Minimal effects were found with all formulations. Thus, the reported side effects may have been associated with the presence of a suppository formulation in the rectum, the process of blood collection, and unfamiliarity with suppository administration. As mentioned above, one subject expelled different formulations on two separate occasions, but expulsion was at times well past the expected peak level of drug serum in the time profile for cefoxitin.

**Microbiological tests.** No *C. difficile* was grown from any of the stool samples. The control *C. difficile* organism grew consistently in all control tests. No bowel pathogens were isolated. Cefoxitin-resistant organisms, mainly *Streptococcus faecalis* (MIC > 125 µg/ml) but also *Bacillus thetaotamicon* (MIC = 64 µg/ml), *Clostridium innocuum* (MIC > 125 µg/ml), *Pseudomonas aeruginosa* (MIC > 125 µg/ml), and *Candida* sp. (MIC > 125 µg/ml), were isolated from many of the stool samples after drug administration. Cefoxitin-resistant organisms (particularly *S. faecalis*) were also isolated in some stool samples obtained before drug administration, especially for those samples collected later in the test series after previous exposure to cefoxitin.

Four of the subjects showed cefoxitin-resistant flora after i.v. administration. The appearance of resistant species did not seem to be related to the extent of absorption of cefoxitin

or the dose, for example, three subjects showed cefoxitin-resistant organisms after study 2, whereas four subjects showed cefoxitin-resistant organisms after studies 4, 5, and 6. Thus, it appears that the selection of cefoxitin-resistant bowel flora after the administration of various suppository formulations of the drug is similar to that found for i.v. administration of cefoxitin.

## DISCUSSION

The results of this investigation confirm results of earlier studies in animals and show that the use of the formulation adjuvants sodium salicylate and Brij 35 (a nonionic surfactant) can greatly enhance the absorption of cefoxitin from the rectum when administered by suppository. A nonoptimized formulation comprising 1.05 g of cefoxitin, 0.5 g of sodium salicylate, and 0.075 g of Brij 35 provided ca. 20% of the i.v. dose and gave a serum concentration-time profile similar to that after i.m. administration of 0.5 g of sodium cefoxitin. The salicylate is absorbed together with the cefoxitin, and the quantity of salicylate used as adjuvant has a definite effect on the resultant bioavailability. The suppositories were well tolerated and there was no local irritation. Studies on bowel flora show that the effect of rectally administered cefoxitin is similar to that after i.v. administration.

The enhanced rectal absorption of cefoxitin by the use of adjuvants provides an opportunity to administer a cephamycin antibiotic by a nonparenteral route.

Further studies are now in progress with a view to optimizing the formulation to give a further increase in bioavailability. Suppository formulations containing adjuvants could also have potential use for the delivery of other poorly absorbed compounds, including antibiotics and peptides.

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